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Original Article

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Uptake of tobacco smoke constituents on exposure to environmental tobacco smoke (ETS)

G. Scherer, C. Conze, A.R. Tricker, and F. Adlkofer

Analytisch-biologisches Forschungslabor Prof. F. Adlkofer, München

Summary. For the purpose of risk evaluation, passive smoking is frequently regarded as low-dose cigarette smoking. However, since the physical, chemical and biological properties of mainstream smoke (MS), which is inhaled by the smoker and environmental tobacco smoke (ETS), which is breathed by the passive smoker are quite different, risk extrapolation from active smoking to passive smoking is of doubtful value. In a series of experimental exposure studies we compared the uptake of tobacco smoke constituents by active and passive smoking. The results show that biomarkers which were found to be elevated after experimental ETS exposure, such as nicotine and cotinine in plasma and urine as well as thioethers in urine, indicate gas-phase exposure in passive smokers, but particle-phase exposure in active smokers. Biomarkers which should indicate the uptake of particle-bound, genotoxic substances with ETS, such as urinary mutagenicity, metabolites of polycyclic aromatic hydrocarbons (PAH) and DNA adducts, were not found to be elevated even after extremely high ETS exposure. From these results we conclude that a risk evaluation for passive smoking on the basis of dosimetric data is currently not possible.

Key words: Environmental tobacco smoke (ETS) – Passive smoking – Smoking – Biomonitoring – Gas phase – Particle phase – Genotoxic substances

Abbreviations: 1-ABP = 4-aminobiphenyl; BaA = benzo(a)anthracene; BaP = benzo(a)pyrene; BE = butanol extraction; BeP = benzo(e)pyrene; CO = carbon monoxide; COHb = carboxyhaemoglobin; DABS = DNA binding substances; DRZ = diagonal radioactive zone; ETS = environmental tobacco smoke; GC = gas chromatography; GC/MS = gas chromatography coupled to mass spectrometry; HPLC = high performance liquid chromatography; HPMA = 3-hydroxypropylmercapturic acid; MS = mainstream smoke; NNK = 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone; NNN = *N*-nitrosonornicotine; NO_x = nitrogen oxides (NO/NO_2); PAH = polycyclic aromatic hydrocarbons; PhIP = 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine; P1 = nuclease P1; RSP = respirable particles

Chronic exposure of non-smokers to environmental tobacco smoke (ETS), also termed passive or involuntary smoking, has been associated with health damage, in particular lung cancer [26, 70]. The evidence for a causal relationship is rather weak, and primarily based on epidemiological findings and linear risk extrapolations from smokers to passive smokers. Meta-analyses of more than 20 epidemiological studies show a relative lung cancer risk of 1.1–1.3 for non-smokers living with a smoking spouse as compared with those living with a non-smoking spouse [37]. Since such low risks are highly sensitive to bias and confounding factors, which are not eliminated by meta-analysis [15, 69], it is unlikely that the controversy can be solved by epidemiological investigations. Risk extrapolation from active to passive smoking simply assumes that passive smoking is a kind of low-dose cigarette smoking [70]. Doses are expressed as 'cigarette equivalents' based on single tobacco-smoke constituents such as nicotine or its major metabolite cotinine [56] or respirable particles [55]. These approaches have been criticized, mainly due to physical, chemical and biological differences between MS and ETS [9].

Table 1 summarizes some major differences between active and passive smoking. In contrast to smokers, passive smokers breath aged tobacco smoke. In vitro tests suggest that aged ETS is less cytotoxic than fresh MS inhaled by the smoker [65]. The smaller size of ETS particles as compared with MS particles [26, 70] and the differences in inhalation patterns between passive and active smoking lead to much lower particle deposition rates of 11% in passive smokers [21] as compared with 50–90% in smokers [22]. Furthermore, the intact clearing mechanism of the respiratory tract of non-smokers removes particles more effectively than the respiratory tract of smokers which may become damaged due to long-term cigarette smok-

Table 1. Differences between active and passive smoking

Parameter (Reference)	Active smoking	Passive smoking
Age of smoke	Fresh	Aged
Cytotoxicity (65)	High	Low
Particle size (70)	0.2–0.4 µm	0.1–0.2 µm
Inhalation pattern	Intermittent deep bursts	Continuous normal breathing
Particle deposition (21, 22)	50–90%	11–15%
Clearing mechanism (41)	May be damaged	Intact
pH of smoke (33, 70)	6.0–6.2	6.7–7.5
Enzyme induction (46, 54)	High	Negligible

ing [41]. ETS is more alkaline than MS [34], and this results in an increased absorption of nicotine in the oral cavity [28]. Toxifying and detoxifying enzymes have been found to be induced in smokers [46, 54]. Whether this is beneficial or harmful for the exposed individual is not known. Finally, risk extrapolation to very low doses is extremely uncertain since the shape of the dose–response curve in this range is based on assumptions and not on experimental or epidemiological data [12].

Estimates for the uptake of gas-phase and particulate-phase constituents by active and passive smoking are presented in Table 2 based on the different deposition rates for smoking and ETS exposure [21, 22]. The theoretical uptake of particle-bound substances is 2–3 orders of magnitude higher in smokers than in passive smokers, whereas the ratio is about 5 for gas-phase constituents under extreme exposure to ETS. This could be of importance with respect to lung cancer risk by passive smoking, considering the fact that the tumorigenic effect of tobacco smoke is mainly attributed to the particle phase [26].

In a series of experimental studies, we quantified the uptake of tobacco smoke constituents by active and passive smoking in order to verify these theoretical considerations. In addition to the most common biomarkers for tobacco smoke exposure such as carboxyhaemoglobin (COHb), nicotine and cotinine in body fluids, we were particularly interested in the uptake of genotoxic substances and their biological effects in the human organism. Furthermore, our efforts were aimed at determining whether or not passive smoking can be regarded simply as low-dose cigarette smoking for risk evaluation.

Methods

Study protocols

Study protocols have been described in detail elsewhere [2, 62–64]. All subjects were healthy male volunteers aged 20 to 40 years. For each study they stayed in the laboratory from the evening of the first experimental day until the morning after the last experimental day. During the study period, smokers were not allowed to smoke except during the special smoking or ETS exposure sessions. These sessions usually last for 8 h and took place in an unventilated, furnished room with five smoking and five non-smoking subjects present. The smokers smoked cigarettes of their usual brand to prescribed schedules. Sham-exposure sessions (control days) were performed in exactly the same way without smoking. During the studies, subjects

Table 2. Estimated uptake doses by active and passive smoking[a]

Tobacco smoke constituents	Smoking (S) (20 cig/d)	Passive smoking (PS) (8 h/d)	Dose ratio S/PS
Gaseous phase			
CO (mg)	40–400	14.4–96	2.7–4.2
Formaldehyde (mg)	0.4–1.8	0.08–0.4	4–5
Volatile nitrosamines (µg)	0.05–1.0	0.03–0.4	1.5–2.5
Benzene (µg)	200–1200	40–400	3–5
Particulate matter			
Particles (mg)	75–300	0.025–0.24	1250–3000
Nicotine (mg)[b]	7.5–30	0.08–0.4	75–90
BaP (µg)	0.15–0.75	0.001–0.011	70–150
Cadmium (µg)	1.5	0.001–0.014	110–1500
Tobacco-specific nitrosamines (µg)	4.5–45	0.002–0.010	2300–4500

[a] Data are compiled according to reference 62

[b] Nicotine is particle-bound in MS and a gas-phase constituents in ETS [14]

ate a defined diet low in PAH [39], which was identical in quality and quantity on each day of the investigations. Urine (24 h) was collected from all subjects beginning at 8 a.m. on each day. Blood was drawn into heparinized tubes immediately before and after each experimental session.

Study 1 [62] comprised four experimental days with eight smokers taking part. Day 1 and day 3 were control days without smoking. On day 2 each subject smoked 24 cigarettes in 8 h (1 cigarette/20 min) through an empty filterholder. On day 4 the same number of cigarettes were smoked with a glass-fibre filter in the holder which removed >99% of the smoke particles. During the smoking sessions, the room was adequately ventilated so that ETS exposure was minimal.

In *Study 2* [62], five smokers and five non-smokers took part over 5 days. On day 1 no smoking, ETS exposure or sham exposure took place. Day 2 and day 4 were control days with 8-h sham-exposure sessions. On day 3 the non-smokers were exposed to the gas phase of ETS for 8 h. For this purpose the non-smokers wore masks equipped with filters (Sekur Polimask-PC, filter classes P1 and P2, Pirelli, Germany), which retained >99% of the respirable particle mass in the inspired air. The smokers who generated the ETS each smoked 24 cigarettes in 8 h (1 cigarette/20 min) and were not exposed to ETS as they smoked cigarettes outside the room through a glass wall with the lit end of the cigarette positioned inside the ETS exposure room. The exhaled smoke was blown through a one-way valve into the exposure room. In order to have the same occupancy, five additional volunteers sat in the experimental room. On day 5 the non-smokers wore no face masks and were thus exposed to whole ETS. The smokers smoked in the experimental room.

Study 3 [63] comprised four experimental days with five smokers and five non-smokers. Day 1 and day 3 were control days. On day 2 and day 4, the non-smokers were exposed to whole ETS generated by the five smokers who each smoked 24 cigarettes during 8 h. On day 4 the smokers smoked through glass-fibre filters and were thus exposed to only the gas phase of MS.

Study 4 [2] had only one experimental day during which eight non-smokers were exposed to the ETS of two smokers for 9 h. Smoking frequency was adusted so that CO concentration fluctuated around 10 ppm.

Study 5 [64] was identical to study 3. However the smokers smoked the whole MS on both exposure days.

Air monitoring

Air sampling tubes were installed at the breathing height of a seated person at the end of the room opposite the seated smokers. CO was measured continuously by an infrared CO monitor (UNOR 6N) (Fa. Maihak, Hamburg, FRG). Nitrogen oxides (NO/NO_2) were detected by chemoluminescence using a Nitrogen-Oxide Analyzer, Model 8840 (Monitor Labs Inc., USA). Nicotine was determined according to the method of Odgen [43]. The alkaloid was absorbed on XAD-4 resin with an air flow rate of 1 l/min. 3-Vinylpyridine was determined in the same samples as nicotine [11]. Sampling times were 4 h on the sham-exposure days and 2 h on the exposure days. Carbonyl compounds (aldehydes) were sampled on Sep-PAK C_{18} (Waters Associates, Milford, Mass., USA) coated with 2,4-dinitrophenylhydrazine and determined by HPLC [35]. Flow rates and sampling times were similar to those for nicotine. Respirable particles (RSP) were determined gravimetrically according to the method of Conner [8]. Solanesol was determined by HPLC in methanol extracts of the filter pads used for particle sampline [45]. The sampling flow rate was 1.5–2.0 l/min. Sampling periods were similar to those for nicotine. PAH were detected according to the method of Grimmer et al. [17]. Benzene was measured by a modification of the NIOSH method [42]. Sampling times ranged from 10 to 60 min with flow rates ranging from 0.5 to 2.0 l/min. The tobacco-specific nitrosamines *N*-nitrosonornicotine (NNN) and 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) were determined by GC/thermal energy analyser detection after sample clean-up by column chromatography [33].

Biomonitoring

COHb was determined spectrophotometrically on fresh blood samples with an IL 182 CO-Oximeter (Intrumentation Laboratories Ltd, USA). Nicotine was determined in plasma and urine by gas chromatography [20] and cotinine in plasma and urine by a modified radioimmunoassay method [36]. Thioethers were measured in urine by quantifying the sulphhydryl groups with Ellman's reagent after alkaline hydrolysis [3, 19]. Sulphur-containing acid compounds in urine were profiled by capillary GC with S-specific detection of the methyl esters with

and without silylation following clean-up by extraction with ethylacetate and column chromatography. 3-Hydroxypropylmercapturic acid (HPMA) was quantified with an S-specific detector after a similar clean-up procedure. Hydroxyphenanthrenes and hydroxypyrenes in urine were analysed by enzyme hydrolysis, extraction into *n*-hexane and clean-up by HPLC fractionation prior to detection as silylated derivatives using GC/MS. Urinary mutagenicity was determined as previously described using the *Salmonella typhimurium* (TA 98)/microsome assay [62]. DNA adducts in lymphocytes were determined by the ^{32}P-postlabelling assay as previously described [24]. Lymphocytes were isolated from the supernatant of white blood cell cultures [24]. DNA binding substances (DABS) in the particle fractions of MS and ETS as well as in urine extracts were detected by ^{32}P-postlabelling of digested DNA isolated from in vitro incubations (37° C, 1 h) of calf thymus DNA with hepatic S9-mix of aroclor-induced rats and cofactors [5, 64].

Statistical analysis

Paired samples (exposed vs non-exposed) were analysed using the Student's *t*-test. For blood and plasma parameters, the level in the respective morning sample before the start of exposure was used as the non-exposed reference value. Similarly, 24-h urine of the previous control day was used as the non-exposed reference value.

Results and discussion

Exposure levels

The results for air monitoring in the experimental room in study 2 are shown in Table 3. ETS components are divided into particle-bound and gas-phase constituents. Except for gas-phase phenanthrene and pyrene, significant increases were measured on the days with experimental ETS exposure (day 3 and 5) as compared with the sham-exposure days (days 2 and 4). The concentrations for the most frequently used ETS markers such as RSP (3–4 mg/m^3), CO (24 ppm) and nicotine (71 µg/m^3) were 10 times higher than those found in everyday environments where real-life exposure to ETS may occur. For example, typical RSP concentrations in real-life rooms where smoking takes place range from 0.05 to 0.35 mg/m^3 [44, 67].

Table 3. Air monitoring in the experimental room during study 2. Data are time-weighted averages for the 8-h exposure sessions. Days 2 and 4 were sham-exposure sessions, and during day 3 and day 5 sessions a total of 120 cigarettes were smoked

	Day			
	2	3	4	5
Particulate matter				
RSP (µg/m^3)	77	3181	78	4091
BaP (ng/m^3)	0.2	21.5	0.3	26.7
BeP (ng/m^3)	0.8	21.5	0.8	24.9
Coronene (ng/m^3)	0.1	2.8	0.6	2.2
Anthanthrene (ng/m^3)	0.06	3.9	0.07	3.1
Benzfluoranthenes (b+j+k) (ng/m^3)	2.1	52.3	1.7	55.3
Chrysene (ng/m^3)	1.8	54.2	1.5	70.5
BaA (ng/m^3)	2.1	18.9	1.1	26.2
Phenanthrene (ng/m^3)	3.7	6.8	1.8	7.4
Pyrene (ng/m^3)	0.6	17.6	0.7	20.5
NNN (ng/m^3)	1	4	1	5
NNK (ng/m^3)	1	9	1	6
Gaseous phase				
CO (ppm)	1.4	24	2.0	24
NO_x (ppb)	38	422	56	449
Formaldehyde (µg/m^3)	3	48	3	49
Acetaldehyde (µg/m^3)	290	1450	85	1390
Propionaldehyde (µg/m^3)	15	120	14	120
Nicotine (µg/m^3)	4	71	6	71
Benzene (µg/m^3)	8	190	12	206
Phenanthrene (ng/m^3)	138	154	nd	258
Pyrene (ng/m^3)	29	24	nd	25

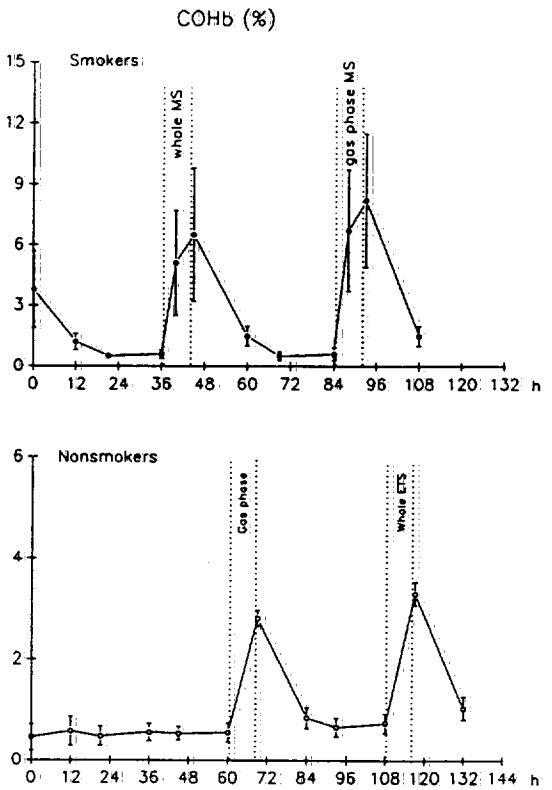

Fig. 1. Time-courses of carboxyhaemoglobin (COHb) for smokers (study 1) and non-smokers (study 2). Data are means with standard deviation bars

Therefore, ETS exposure levels in our studies have to be regarded as purely experimental.

More recently, 3-vinylpyridine has been proposed as a gas-phase marker for ETS [11] and solanesol as a particle-phase marker for ETS [44]. In study 5 we measured both ETS components. 3-Vinylpyridine concentrations were 0 and 15 µg/m^3 on the control and exposure days, respectively. For solanesol the respective levels were 0 and 110 µg/m^3. Solanesol concentrations under real-life conditions range form 6.4 to 12.8 µg/m^3 [44].

Biomonitoring

In the following sections the uptake of different tobacco smoke constituents after experimental ETS exposure are compared with those after active smoking. For some substances, the experimental design allows differentiation between uptake as gas-phase or as particle-bound substances [62].

Carboxyhaemoglobin (COHb). The time-courses of COHb for smokers and ETS-exposed non-smokers are shown in Fig. 1. As expected, increases after gas-phase and whole MS or ETS exposure are similar. The reasons for the somewhat higher COHb levels after gas-phase MS smoking as compared with whole MS smoking and whole ETS exposure as compared with gas-phase ETS exposure have been discussed previously [62]. Under the experimental conditions applied, the mean increase in COHb after smoking (6.5–8.2%) is three times larger than that after ETS exposure (2.5%), which is in agreement with the estimated uptake ratio (Table 2) considering that the experimental conditions involved extremely high exposure to ETS containing 24 ppm CO for 8 h. Under real-life ETS exposure, no increase, or only a marginal increase, in COHb concentration is observed [29, 68], which again demonstrates that extremely high ETS doses were applied. Due to its low sensitivity and lack of specificity, COHb is not a suitable biomarker for everyday ETS exposure.

Nicotine and cotinine. The uptake of nicotine was monitored by determining nicotine (Fig. 2) and cotinine (Fig. 3) in both plasma and urine. The results clearly show that smokers take up nicotine in particle-bound form. In contrast to this, nicotine is taken up as a gas-phase constituent from ETS (Fig. 2). Due to the reduced capacity of non-smokers to metabolize nicotine to cotinine and the long biological half-lives of both these compounds, higher amounts of cotinine are found in plasma and urine on the days after exposure of non-smokers to gas phase and whole ETS than on the actual exposure days (Fig. 3). These findings are in agreement with analytical data showing the nicotine in MS is particle-bound, whereas it is nearly exclusively found in the gas phase of ETS [14]. Therefore, nicotine and cotinine in body fluids are biomarkers for the uptake of smoke particles in active smokers but indicate ETS gas phase exposure in passive smokers. Comparison of cotinine levels in body fluids of active and passive smokers for the purpose of risk estimation, as performed by Russell [56], is therefore misleading. On the other hand, cotinine in plasma, urine and saliva is presently the most reliable biomarker for ETS exposure [29], as long as comparisons are limited to subjects with different degrees of ETS exposure. Extrapolation from the uptake of nicotine to that of other tobacco smoke constituents is of doubtful validity due to a more rapid removal of nicotine than other components from ETS in indoor environments [11].

Thioethers (mercapturic acids). The urinary excretion of thioethers is regarded to be an indicator

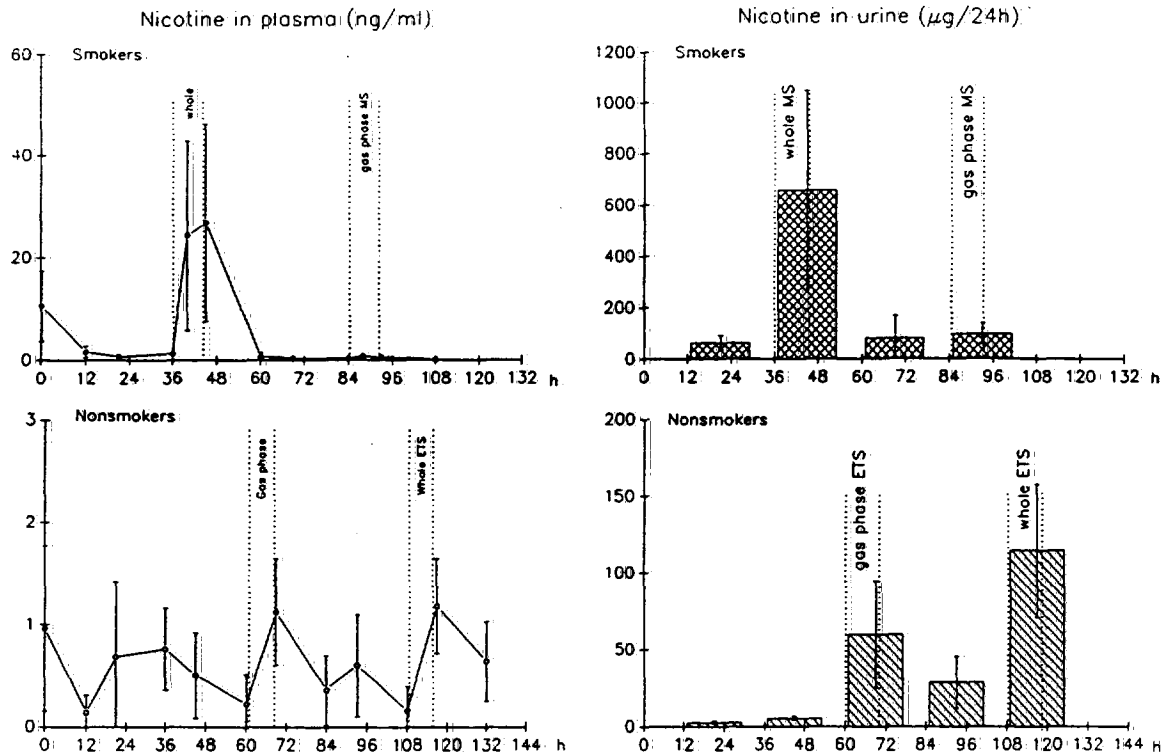

Fig. 2. Time-courses of plasma nicotine (*left*) and urinary excretion of nicotine (*right*) for smokers (study 1) and non-smokers (study 2). Data are means with standard deviation bars

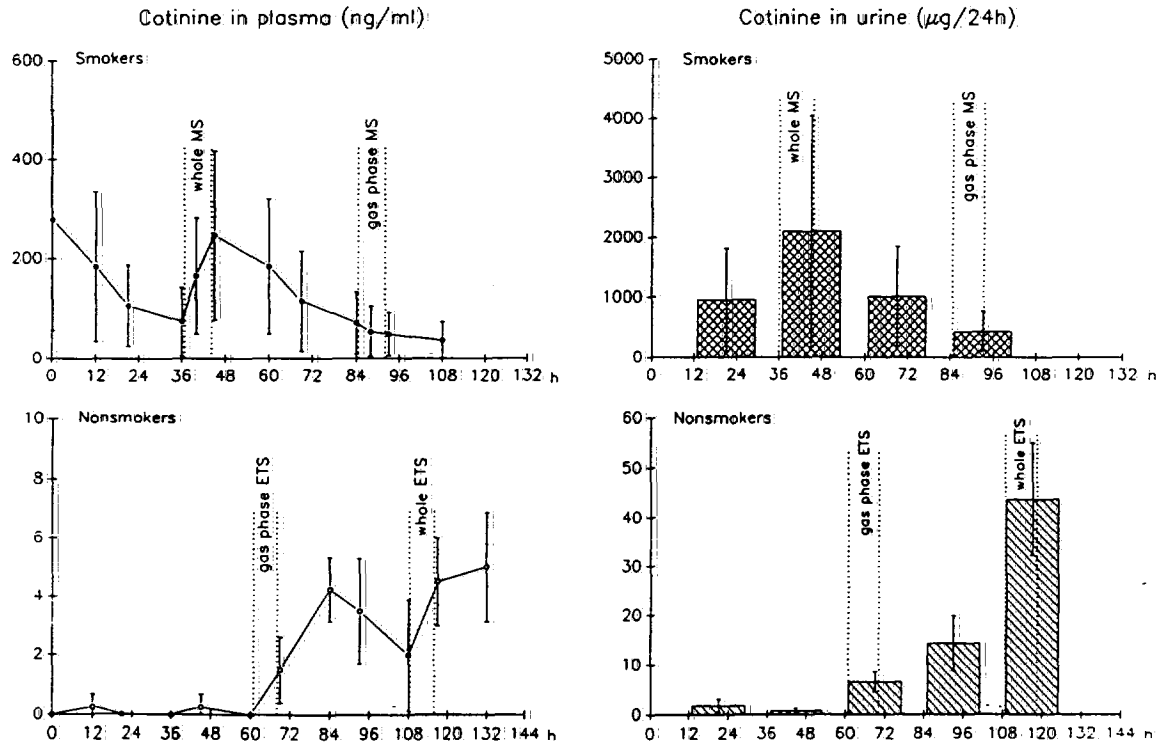

Fig. 3. Time-courses of plasma cotinine (*left*) and urinary excretion of cotinine (*right*) for smokers (study 1) and non-smokers (study 2). Data are means with standard deviation bars

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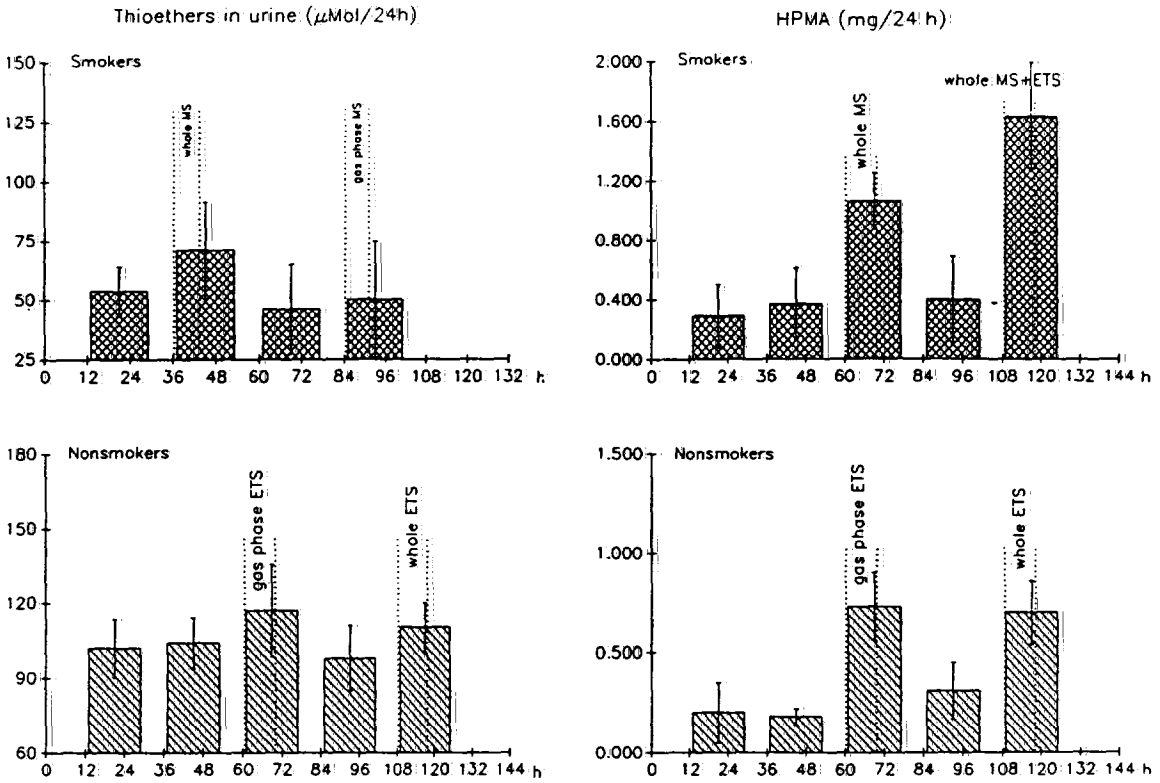

Fig. 4. *Left*. Urinary excretion of thioethers by smokers (study 1) and non-smokers (study 2). *Right*. Urinary excretion of 3-hydroxypropylmercapturic acid (HPMA) by smokers (study 2) and non-smokers (study 2). Data are means with standard deviation bars

of the exposure to electrophilic substances [6]. The intracellular glutathione-S-alkyl-transferase/-glutathione system protects the organism from the destructive effects of electrophiles by binding them to the free SH-group of glutathione. Smoking has been shown to increase thioether excretion [71]. We have confirmed this in a previous study [61]. In addition, we also found a significant increase in thioether excretion by non-smokers after high ETS exposure. In more recent studies [62], we showed that the elevated thioether excretion by smokers is primarily caused by uptake of MS particles, whereas in ETS-exposed non-smokers the increase in thioether excretion is due to only the gas phase of ETS (Fig. 4).

The tobacco smoke constituents responsible for thioether formation are unknown. In a first attempt to characterize and identify tobacco smoke-related thioethers, we profiled the sulphur-containing acids (assumed to contain the majority of mercapturic acids) in the urine of smokers and non-smokers with and without smoking or ETS exposure, respectively. In most of the subjects, the number and intensity of the S-containing peaks in the gas chromatograms increased after tobacco smoke exposure. However, identification of specific mercapturic acids turned out to be very difficult in these complex mixtures. We were able to identify 3-hydroxypropylmercapturic acid (HPMA) in urine of smokers and ETS-exposed non-smokers. The elevation of HPMA in urine of passive smokers is exclusively caused by the gas phase of ETS (Fig. 4). No assertion for smoking with respect to HPMA being formed by gas- or particle-phase constituents of MS can be made, since HPMA was not determined in study 1. It is not clear which tobacco smoke constituents are responsible for the in vivo formation of HPMA. After application of acrolein to rats, HPMA has been reported to be a major metabolite in urine [32]. However, this was not confirmed in a more recent investigation [10].

The elevation of non-specific urinary thioethers following exposure of active smokers to particulate matter and passive smokers to gas-phase ETS again shows that differences exist between active

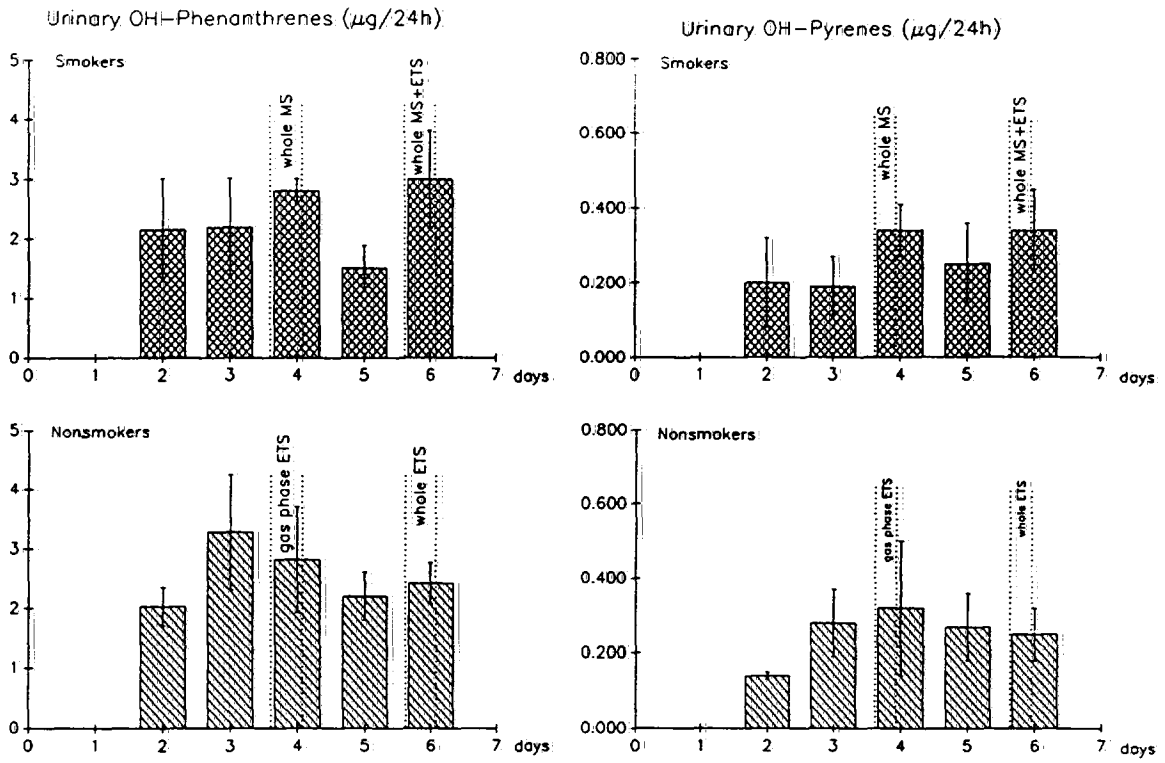

Fig. 5. *Left.* Urinary excretion of hydroxyphenanthrenes by smokers (study 2) and non-smokers (study 2). Hydroxyphenanthrenes are the sum of 1-, 2-, 3-, 4- and 9-OH-phenanthrene. *Right.* Urinary excretion of hydroxypyrenes by smokers (study 2) and non-smokers (study 2). Hydroxypyrenes are the sum of 1- and 2-OH-pyrene. Data are means with standard deviation bars

smoking and exposure to ETS, and an extrapolation from active to passive smoking cannot be made.

In general, the determination of urinary thioethers is not a suitable method for biological monitoring of tobacco smoke exposure. Apart from the low sensitivity and the lack of specificity, the main reason is that changes in thioether excretion can only be detected when the diet is carefully controlled as performed in our studies [2, 61, 62].

Polycyclic aromatic hydrocarbons (PAH). PAH represent a class of carcinogens in tobacco smoke which has been intensively investigated. Their importance in tobacco smoke carcinogenesis has previously been rated high [18]. More recently, other carcinogens, such as nitrosamines and free radicals, as well as cocarcinogens and promoters, are assumed to be of similar, or even greater, importance in tobacco smoke carcinogenesis [26]. Hydroxyphenanthrenes [23] and hydroxypyrenes [30] in urine have been proposed as biomarkers for the uptake of PAH. Phenanthrene is primarily a gas-phase constituent of ETS, the background concentration is relatively high as can be seen from the level of 138 ng/m^3 observed on day 2 of study 2, when no smoking took place in the experimental room (Table 3). ETS exposure, either with gas-phase or whole ETS, does not lead to a measurable increase in urinary hydroxyphenanthrene excretion (Fig. 5). After smoking, a small but significant increase was found (Fig. 5). In everyday passive smoking, ETS-related uptake of phenanthrene (and PAH in genral) can be neglected, particularly when the diet as an important source for PAH uptake is considered [39].

The same conclusion can also be drawn from the findings with urinary hydroxypyrene excretion after smoking and ETS exposure (Fig. 5). A small but significant increase is observed after smoking. The room air measurements show pyrene concentrations in the gas phase of about 25–30 ng/m^3, which are unrelated to the actual smoking taking place in the room (Table 3). The particle-bound pyrene levels increased from <1 ng/m^3 without smoking to 18–20 ng/m^3 with smoking (Table 3). However, this increase in particle-bound pyrene did not lead to a measurable elevation in urinary

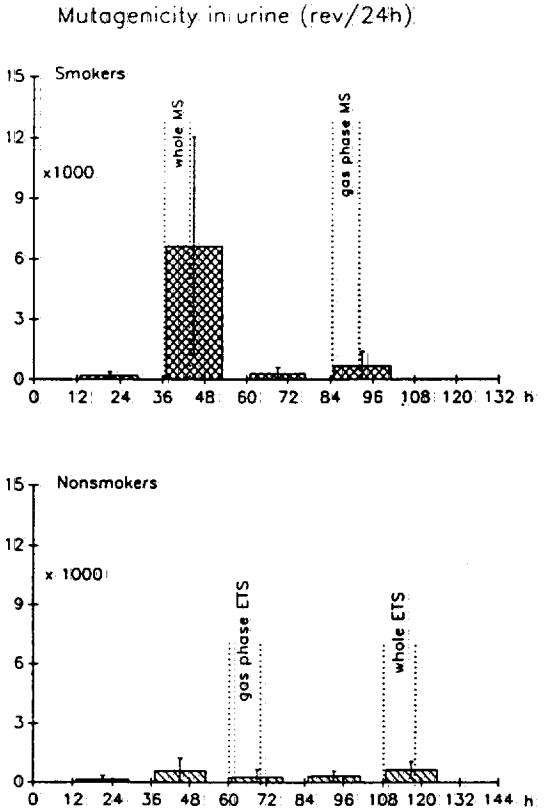

Fig. 6. Urinary mutagenicity in smokers (study 1) and nonsmokers (study 2). Data are means with standard deviation bars

hydroxypyrene excretion after whole ETS exposure, indicating that either the background body burden of pyrene was too high and/or uptake of ETS particle phase was too low. The latter explanation is in accordance with the low deposition rate of ETS particles in the respiratory tract during passive smoking [21].

Determination of PAH metabolites in urine is not sensitive enough for biomonitoring of ETS exposure under real-life conditions. The data shown here and those from our earlier studies [1, 23, 29] suggest that passive smoking only marginally contributes to the everyday PAH exposure. General air pollution and diet are the major exposure sources to PAH.

Mutagenicity. A more general approach for determining the uptake of genotoxic substances is the measurement of urinary mutagenicity in exposed subjects. Increased mutagenicity in smokers' urine has been found in many studies [26]. Our results with active smokers clearly show that the urinary mutagenicity is caused nearly exclusively by the uptake of MS particulate matter (Fig. 6). Whether ETS exposure can lead to an elevated urinary mutagenicity is a matter of controversy. In most investigations no significant increase has been observed [25, 31, 60, 66]. In two studies [4, 40], with experimental ETS exposures comparable to those in our investigations [60, 62], a significant elevation in urinary mutagenicity was found. In study 2, urinary mutagenic activity after gas-phase and whole ETS exposure were within the normal fluctuations of the method (Fig. 8) [62]. There is sufficient evidence that ETS particles are mutagenic [38]. In ETS about 10% [57], and in sidestream smoke (SS) about 20% [7], of the mutagenic activity has been found in the vapour phase. Therefore, it can be assumed that the mutagenic activity of ETS is primarily particle bound.

Based on the particle concentration occurring during experimental ETS exposure (Table 3), a breathing rate of 0.5 m^3/h and a deposition rate of 11% [21], it can be estimated that the nonsmokers in study 2 could have taken up about 1.8 mg of ETS particles during exposure on day 5 compared with about 300 mg of MS particles taken up the smokers smoking 24 cigarettes. Assuming similar specific mutagenic activities for ETS and MS particles (MS particles have been reported to have a specific activity 2–3 times higher than SS particles) [7], ETS exposure in terms of particle exposure would be equivalent to smoking 0.14 cigarettes. In our experience, the Ames test for detection of urinary mutagenicits is far too insensitive to assess such a low exposure. The results of our investigations [60, 62], as well as those of other authors [25, 31, 66], suggest that urinary mutagenicity, which would be a potential marker for ETS particle exposure, remains unchanged after ETS exposure. Taking into consideration dietary factors that may elevate or reduce the mutagenic activity of urine [1, 23, 39, 58], we conclude that this is not a useful marker for the uptake of genotoxic substances in real-life passive smoking.

DNA adducts. DNA adducts are useful biomarkers for the detection of exposure to genotoxic substances at the relevant target, namely the DNA in the cell nucleus. DNA adduct levels are modulated by several factors including exposure dose, metabolic activation and detoxification, survival time of the cells under investigation, as well as stability and repair of the formed DNA adducts. Determination of DNA adducts by the ^{32}P-postlabelling assay is a sensitive method with a detection limit of 1 adduct/10^{10} normal nucleotides [52].

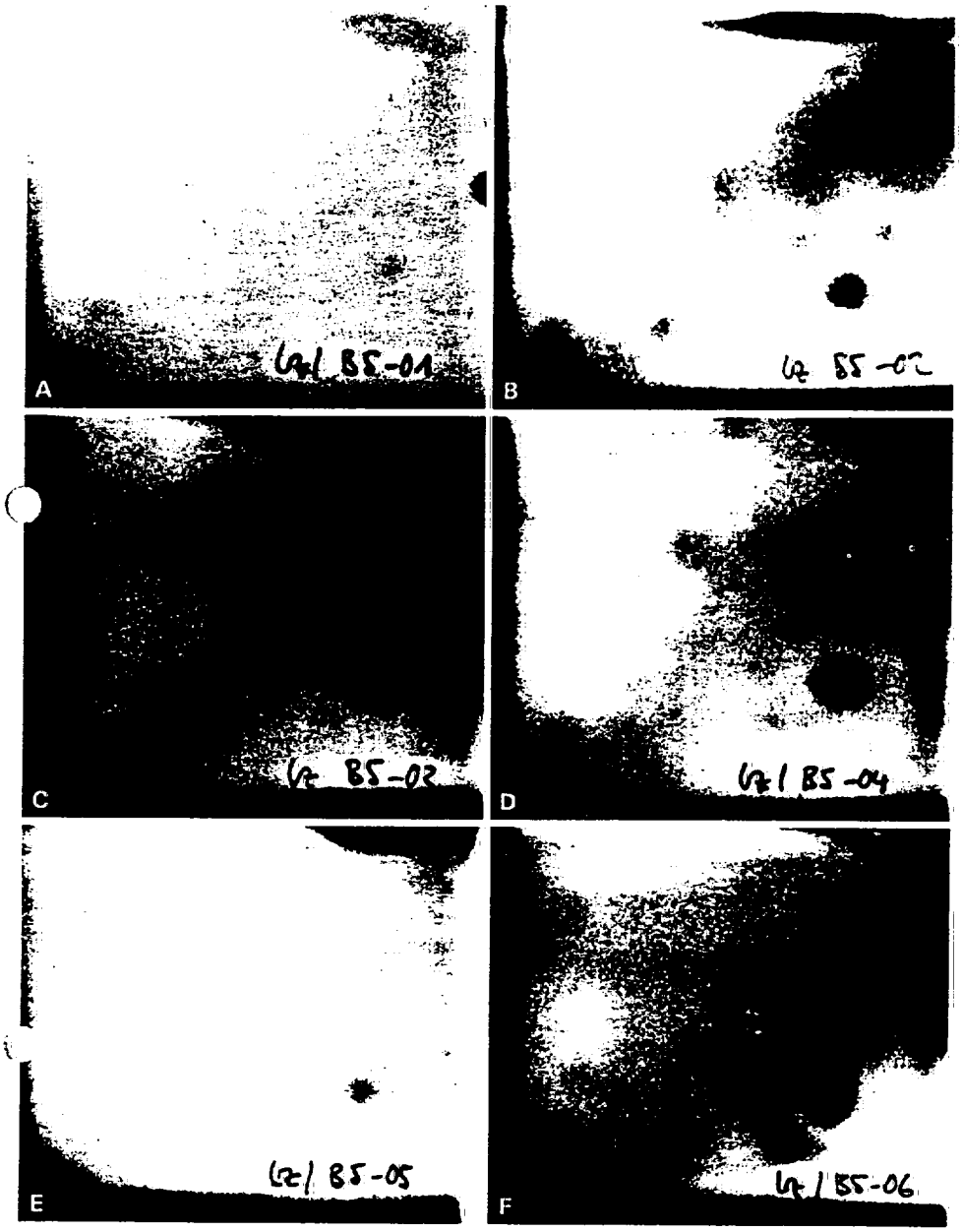

Fig. 7. Autoradiograms of ^{32}P-postlabelled DNA adducts from lymphocytes of five smokers (A–E) and five non-smokers (F–J). Blood samples for lymphocyte isolation were drawn directly after the exposure session on day 2 of study 2. Autoradiography was carried out at −84° C for 100 h

Since the chemical structure of the formed adducts does not need to be known, this method is particularly useful for the detection of DNA adducts after exposure to complex mixtures such as tobacco smoke. The ^{32}P-postlabelling method was applied to DNA of blood monocytes of the subjects in Study 2 [24]. Additional smoking-related DNA adducts were found after smoking 24 cigarettes in 8 h. These adducts disappeared within 36 h after smoking cessation, which is primarily due to the limited life-span of monocytes (about 8 h). No additional adduct spots could be detected after ETS exposure [24]. In a recent study (study 3) with similar tobacco smoke exposures, exposure-related ad-

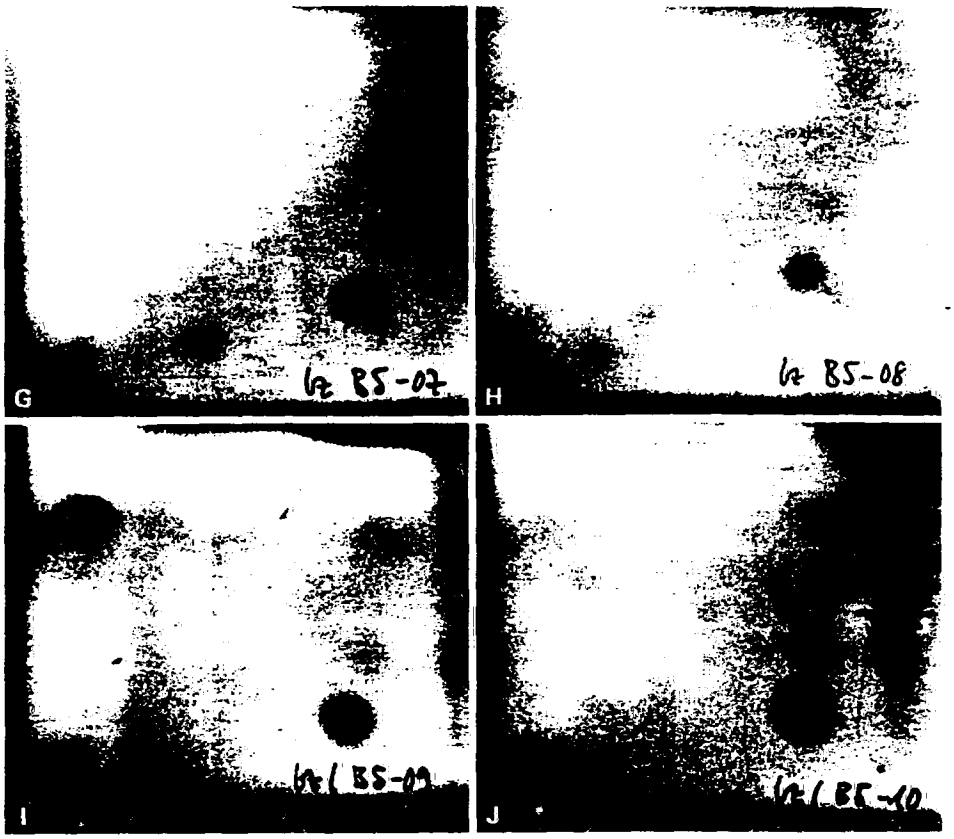

Fig. 7 G–J

ducts could not be identified in monocytes from smokers and non-smokers [63]. However a slightly greater number of adducts were found in monocytes of smokers when smoking the complete MS as compared with smoking the gas phase of MS or being abstinent from smoking. Although these results need further confimation, they are in agreement with the idea that the ^{32}P-postlabelling assay with nuclease P1 enrichment detects bulky aromatic adducts, most probably originating from particle-phase constituents of tobacco smoke. The lack of additional DNA adducts in ETS-exposed non-smokers suggests that the uptake of ETS particles is below the detection limit of this method.

In contrast to DNA adducts in monocytes, DNA adducts in T lymphocytes (~80% of total lymphocytes), which have a life-span of several years, should indicate chronic exposure to DNA adduct-forming compounds. The autoradiograms of five smokers and five non-smokers are shown in Fig. 7. Visual inspection of the spot patterns reveals no characteristic difference between smokers and non-smokers. This qualitative result is in agreement with other reports from the literature [27, 49, 50, 53, 59]. In one study [59], the mean adduct levels in smokers were found to be significantly higher than those in non-smokers. In the same study, a significant intra-individual variation (two blood samples from three smokers were drawn with a 3-week interval) was observed. We made the same observation when analysing four blood samples drawn within 4 days from each subject in study 3.

In our view, the detection of tobacco smoke-related DNA adducts in white blood cells by the ^{32}P-postlabelling method has not been able to reveal conclusive results. We agree with Foiles et al. [16], who regard the lack of information on the structure of the majority of adducts observed by this method in different human tissues as a serious limitation.

In another approach, we tried to detect 'DNA binding substances' (DABS) in complex mixtures such as MS particulate matter, ETS particles and urine extracts of smokers and non-smokers with and without tobacco smoke exposure [64]. These mixtures were incubated with a target DNA and a metabolizing system so that the direct carcinogens and the enzymatically formed ultimate carcinogens could bind to target DNA. It has been sug-

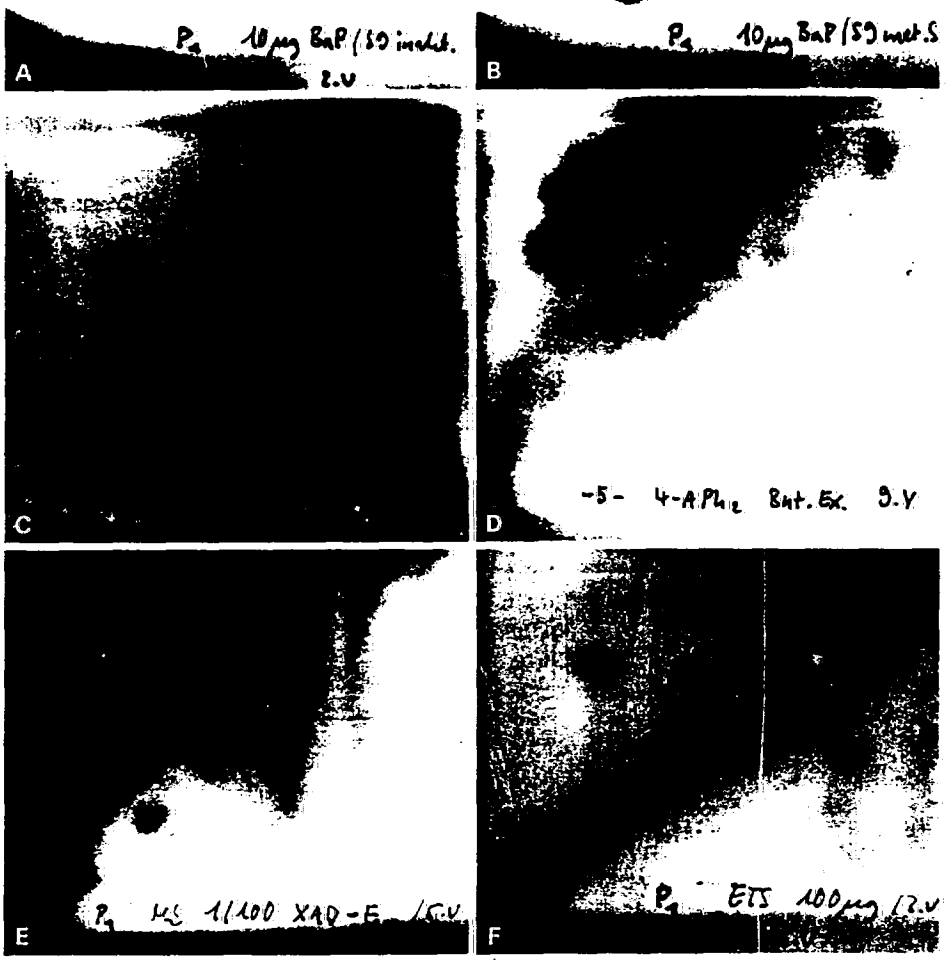

Fig. 8. Autoradiograms of ^{32}P-postlabelled DNA adducts formed after in vitro incubation of benzo(a)pyrene (BaP) (A, B), 4-aminobiphenyl (4-ABP) (C, D), particle phases of mainstream smoke (*MS*) (E) and environmental tobacco smoke (*ETS*) (F) as well as extracts of 100 ml urine from smokers (G, H) and non-smokers (I–L) collected in study 4. All samples except for sample A were incubated with active S9-mix as described in the text. Adduct enrichment was performed by either nuclease P1 treatment (P1) or butanol extraction (BE) as indicated. Autoradiography was carried out at −84° C for 40 h. A BaP (10 μg)+ inactivated S9-mix; B BaP (10 μg)/P1; C 4-ABP (50 μg)/P1; D 4-ABP (50 μg)/BE; E MS (1/100 cigarette)/P1; F ETS (100 μg)/P1; G urine extract of a smoker/P1; H same as G/BE; I urine extract of a non-smoker without ETS exposure/P1; J same as I/BE; K urine extract of non-smoker I after ETS exposure/P1; L same as K/BE

gested that the mutagenic compounds in smokers' urine are heterocyclic aromatic amines [13, 47] of which 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) has been implicated as a major cause of DNA damage [48]. A metabolizing system able to activate these compounds was used [5]. Results obtained with this in vitro assay are shown in Fig. 8. The particulate phases from MS and ETS

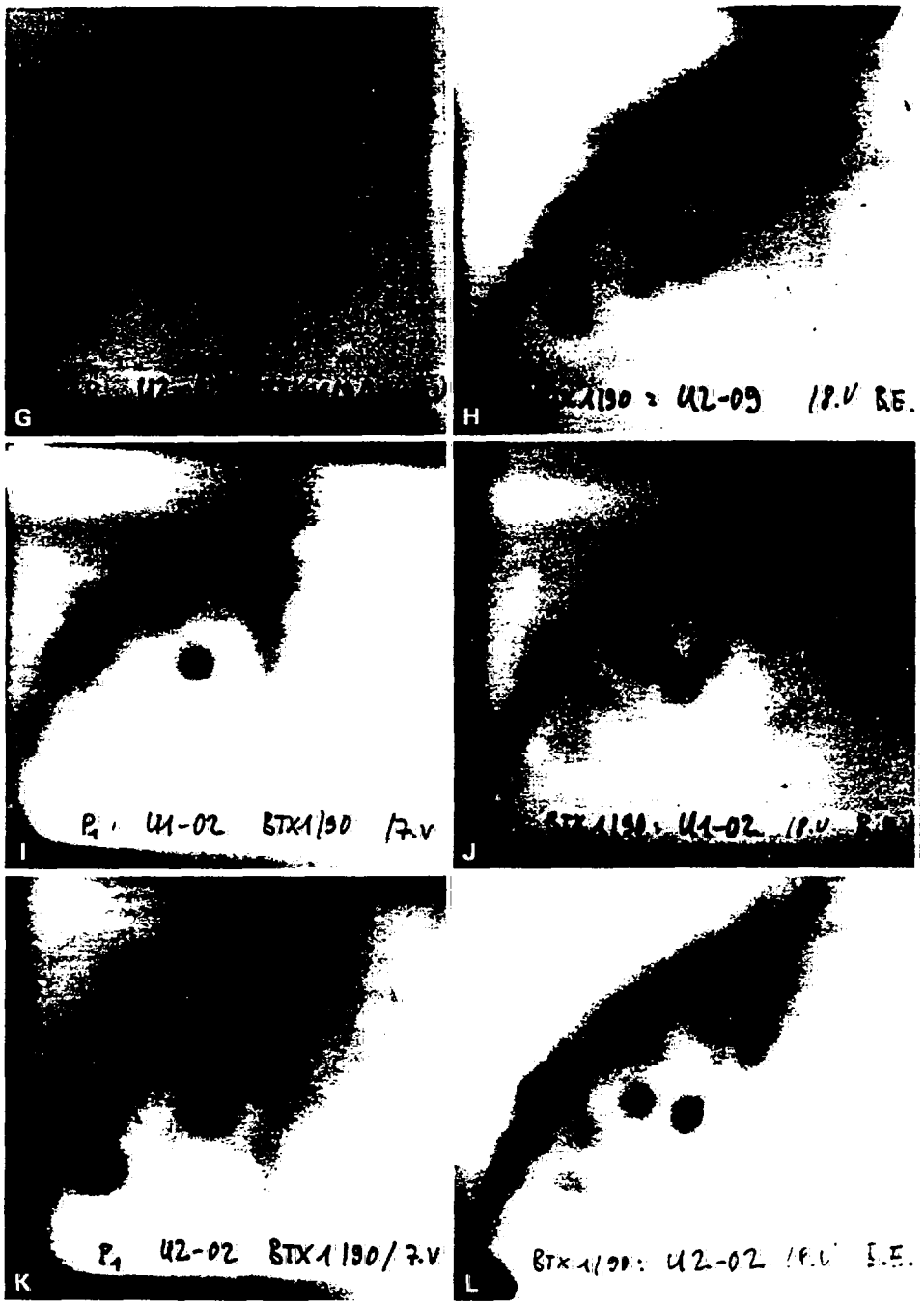

Fig. 8G–L

form diagonal radioactive zones (DRZ) comparable to those observed with DNA from lung, heart, kidney, bladder and oesophagus from smokes [51].

Urine extracts from smokers and non-smokers (either with or without tobacco smoke exposure) showed a couple of DNA adduct spots. The spot pattern varied with the adduct enrichment method (nuclease P1 treatment or butanol extraction). The detected spots were not related to one of the heterocyclic aromatic amines tested in this assay. Fur-

thermore, no DNA adducts could be identified which were exclusively related to smoking or ETS exposure. Since the diet was carefully controlled in these studies, it is unlikely that the observed inter-individual differences in urinary DABS were caused by dietary factors. These results do not agree with those reported by Peluso et al. [47, 48], who found no, or only faint, DNA adduct spots in urine extracts of non-smokers, and adduct spots in urine extracts from smokers of black tobacco suggested to have been caused by PhIP. Investigations are in progress in our laboratory in order to clarify the origin of the DABS in both smokers' and non-smokers' urine extracts.

Conclusions

he small increase in lung cancer risk in non-smokers exposed to other people's tobacco smoke, as suggested by meta-analysis of more than 20 epidemiological studies, is at variance with dosimetric findings observed in exposure studies with smokers and non-smokers. These studies have shown that the uptake of particle-bound substances is predominant in active smoking, whereas uptake of gas-phase substances is predominant in passive smoking. According to present knowledge, the genotoxic potential is primarily located in the particulate matter of both MS and ETS. Currently available biomonitoring methods sensitive enough for detecting ETS exposure only indicate the uptake of gas-phase constituents of ETS. There is no biomarker available which indicates the uptake of ETS particles. Therefore, although low amounts of genotoxic substances can be inhaled by passive smoking, a risk evaluation on the basis of dosimetric data for these compounds is not presently possible.

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Dr. G. Scherer
Analytisch-biologisches Forschungslabor Prof. Adlkofer
Goethestrasse 20
W-8000 München 2, FRG